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Review Article

The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles

Ning Li [a,b,c], Tian Xia [a], Andre E. Nel [a,b,c,d,*]

[a] Division of NanoMedicine, Department of Medicine, University of California, Los Angeles, CA 90095, USA
[b] Asthma and Allergic Diseases Cooperative Research Centers, University of California, Los Angeles, CA 90095, USA
[c] The Southern California Particle Center, University of California, Los Angeles, CA 90095, USA
[d] California NanoSystems Institute, University of California, Los Angeles, CA 90095, USA

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Abstract

Ambient particulate matter (PM) is an environmental factor that has been associated with increased respiratory morbidity and mortality. The major effect of ambient PM on the pulmonary system is the exacerbation of inflammation, especially in susceptible people. One of the mechanisms by which ambient PM exerts its proinflammatory effects is the generation of oxidative stress by its chemical compounds and metals. Cellular responses to PM-induced oxidative stress include activation of antioxidant defense, inflammation, and toxicity. The proinflammatory effect of PM in the lung is characterized by increased cytokine/chemokine production and adhesion molecule expression. Moreover, there is evidence that ambient PM can act as an adjuvant for allergic sensitization, which raises the possibility that long-term PM exposure may lead to increased prevalence of asthma. In addition to ambient PM, rapid expansion of nanotechnology has introduced the potential that engineered nanoparticles (NP) may also become airborne and may contribute to pulmonary diseases by novel mechanisms that could include oxidant injury. Currently, little is known about the potential adverse health effects of these particles. In this communication, the mechanisms by which particulate pollutants, including ambient PM and engineered NP, exert their adverse effects through the generation of oxidative stress and the impacts of oxidant injury in the respiratory tract will be reviewed. The importance of cellular antioxidant and detoxification pathways in protecting against particle-induced lung damage will also be discussed.

Keywords: Particulate matter; Oxidative stress; Asthma; Dendritic cells; Adjuvant effect; Nanotoxicology; Free radicals

Contents

* Corresponding author. Fax: +1 310 206 8107.
E-mail address: anel@mednet.ucla.edu (A.E. Nel).

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Introduction

Increased vehicular traffic and other combustion processes have resulted in a significant increase in ambient particulate matter (PM) over the past two decades. A sudden surge in the level of PM has been linked to increased morbidity and mortality due to cardiorespiratory events, including asthma, chronic obstructive pulmonary disease, and atherosclerosis [1–12]. Both in vitro and in vivo studies of the health effects of ambient PM have identified the generation of oxidative stress as one of the major mechanisms by which air pollution particles exert adverse biological effects. Among particles of different sizes, it has also been established that ultrafine particles (UFP), which have an aerodynamic size of <100 nm, are potentially the most dangerous due to their small size, large surface area, deep penetration and ability to be retained in the lung, and high content of redox-cycling organic chemicals [3]. In addition, increased use of engineered nanoparticles (NP) in a wide range of industries has introduced a potential new type of inhaled particulate pollutant [13]. Examples include carbon black, TiO_2, ZnO, and CeO_2 nanoparticles [13]. Currently, little is known about the potential adverse health effects of these particles. Therefore, there is an urgent need to understand the potential impact of inadvertent ambient (UFP) or engineered NP exposure on human health. None of these NP are currently being regulated. In this communication, we will review the mechanisms by which particulate pollutants, including ambient and engineered NP, exert their deleterious effects through an ability to generate reactive oxygen species (ROS) and oxidative stress. We will discuss the role of oxidant injury by ambient and engineered NP in the respiratory tract. We will also discuss the importance of cellular antioxidant and detoxification pathways in protecting against particle-induced lung damage.

The role of oxidative stress in the health effects of particulate pollutants

Several mechanisms have been proposed to explain the adverse health effects of particulate pollutants. These include inflammation, endotoxin effects, stimulation of capsaicin/irritant receptors, autonomic nervous system activity, procoagulant effects, covalent modification of cellular components, and ROS production [3]. Among these, ROS production and the generation of oxidative stress have received the most attention.

Cellular redox homeostasis is carefully maintained by an elaborate antioxidant defense system, which includes antioxidant enzymes, proteins, and low-molecular-weight scavengers. Excessive ROS production or a weakening of antioxidant defense could lead to oxidative stress [1]. Oxidative stress is a state of redox disequilibrium that is defined as a decrease in the cellular glutathione (GSH)/glutathione disulfide (GSSG) ratio but functionally should be seen as a cellular stress response that activates a number of the redox-sensitive signaling cascades [1]. The GSH/GSSG redox pair not only serves as the principal homeostatic regulator of redox balance but also functions as a sensor that triggers these stress responses that, depending on the rate and level of change in this ratio, could be protective or injurious in nature [1,14].

Using diesel exhaust particles (DEP) as a model air pollutant, a hierarchical cellular response model has been developed to explain the role of oxidative stress in mediating the biological effects of PM [14,15] (Fig. 1). This three-tier model posits that low levels of oxidative stress induce protective effects that may yield to more damaging effects at higher levels of oxidative stress (Fig. 1). The protective effects (Tier 1) are induced by the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which regulates transcriptional activation of >200 antioxidant and detoxification enzymes that are collectively known as the phase 2 response [16,17]. Examples of phase 2 enzymes include heme oxygenase 1 (HO-1), glutathione *S*-transferase (GST) isoenzymes, NADPH quinone oxidoreductase [18], catalase, superoxide dismutase (SOD), and glutathione peroxidase [17,19]. Defects in or aberrancy of this protective pathway could determine the susceptibility to particle-induced oxidant injury, e.g., the exacerbation of airway inflammation and asthma by DEP [1]. Thus, it is important to mention that due to the protective Tier 1 response, particle-induced ROS production does not automatically lead to adverse biological outcomes. Should these protective responses fail to provide adequate protection, a further increase in ROS production can result in proinflammatory (Tier 2) and cytotoxic (Tier 3) effects [3,14]. Proinflammatory effects are mediated by the redox-sensitive MAP kinase and NF-κB cascades that are responsible for the expression of cytokines, chemokines, and adhesion molecules, many of which are involved in the inflammatory process of the lung [1,3,14,19]. Tier 3 cytotoxic effects (aka toxic oxidative stress) involve mitochondria, which are capable of releasing proapoptotic factors and inducing apoptosis of lung cells [20,21]. Taken together, the hierarchical cellular oxidative stress model provides a mechanistic platform against which to understand how PM generate adverse health effects.

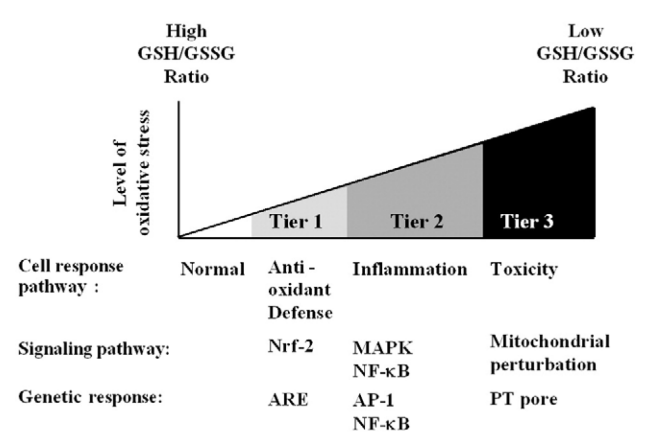

Fig. 1. Hierarchical oxidative stress responses. At a low level of oxidative stress (Tier 1), antioxidant enzymes are induced to restore cellular redox homeostasis. At an intermediate level of oxidative stress (Tier 2), activation of MAPK and NF-κB cascades induces proinflammatory responses, e.g., cytokines and chemokines. At a high level of oxidative stress (Tier 3), perturbation of the mitochondrial permeability transition pore and disruption of electron transfer result in cellular apoptosis or necrosis.

Generation of oxidative stress by ambient particulate pollutants

How is oxidative stress generated by ambient PM? The aerodynamic diameters of ambient particle size vary from 0.005 to 10 μm. Three different types of ambient particles, as defined by size, are characterized in Table 1. Among these, the small size and large surface area of UFP make them carriers for metals and a large number of organic carbon compounds. Many of these PM components are capable of ROS generation, e.g., promotion of Fenton and Haber Weiss chemistry to generate ROS and adverse biological effects [1]. In addition, the redox cycling of organic chemical compounds such as quinones could also give rise to the formation of superoxide radicals ($O_2^{\bullet -}$) [1].

Taking advantage of the Versatile Aerosol Concentration Enrichment Systems, which can collect highly concentrated ambient particles (CAP) of various sizes, the Southern California Particle Center has conducted studies to identify the relative toxicity of coarse, fine, and ultrafine particles in the Los Angeles Basin. The toxic potential of these particles could be correlated with their chemical composition and their capacity to induce oxidative stress [15,22]. In particular, it was demonstrated that the biological activity and oxidant potential of CAP are determined by the content of their redox cycling chemicals [22] (Table 1). Whereas coarse PM include mostly crustal elements, UFP, which are mainly derived from combustion sources, have been shown to contain significantly more organic carbon compounds such as polycyclic aromatic hydrocarbons (PAH) and quinones [22] (Table 1). A strong correlation exists between the PM content of redox-active chemicals and their capability to induce oxidative stress in macrophages and bronchial epithelial cells [22]. Moreover, the intracellular localization of the particles could also play a role in ROS production. For instance, electron microscopy has revealed that UFP are capable of localizing inside damaged mitochondria. Both aromatic organic compounds and quinones contribute to mitochondrial injury and ROS generation [21,22]. Thus, the extent of cellular toxicity is directly related to these organic chemical compounds.

It is possible to assess the pro-oxidant activity of PM by using the dithiothreitol (DTT) assay that reflects the particle content of redox cycling chemical groups such as quinones [22,23]. This assay is premised on the interaction of redox-cycling chemical quinones (Q) with DTT:

$$Q + DTT \rightarrow semi\text{-}Q + DTT - thiyl, \quad (1)$$

$$Q + DTT - thiyl \rightarrow semi\text{-}Q + DTT - disulfide, \quad (2)$$

$$2\ semi\text{-}Q + 2\,O_2 \rightarrow 2\,Q + 2\,O_2^{\bullet -}, \quad (3)$$

$$DTT + 2\,O_2 \rightarrow DTT - disulfide + 2O_2^{\bullet -}\ (net).$$

The loss of DTT can be followed by its reaction with 5,5′-dithiobis-(2-nitrobenzoic acid). This assay provides a convenient means of comparing the pro-oxidative activity of ambient samples collected in an urban environment. In general, DTT activity is highest in UFP, which also correlates with their ability to induce cellular oxidant stress responses such as HO-1 expression [22].

Table 1
Comparison of coarse, fine, and ultrafine particles

Parameter	Coarse	Fine	Ultrafine
Size	2.5–10 μm	0.10–2.5 μm	<0.10 μm
Organic carbon content	+	++	+++
Metal content	+++	++	+
PAH content	+	+	+++
Source of ROS	Transition metals	PAH Quinones	PAH Quinones

Studies using fractionated organic DEP extract have demonstrated that quinones and PAH are representative organic chemical groups that could contribute to oxidant injury in the lung [23–25]. PAH can be converted to quinones via biotransformation, e.g., through reactions involving cytochrome P450 1A1, expoxide hydrolase, and dihydrodiol dehydrogenase [26]. Quinones produce ROS and may be key compounds in PM toxicity along with transition metals [24,26]. Redox-cycling quinones undergo one-electron reductions by NADPH cytochrome P450 reductase to form semiquinones [27]. The semiquinones can be recycled to the original quinones, leading to the formation of $O_2^{\bullet -}$. Not only are quinones by-products of diesel fuel combustion, but they can also be formed by enzymatic conversion of PAH in lung tissue [28].

In addition, it is necessary to point out that other PM chemicals and transition metals may also contribute to ROS overproduction. For example, it has been demonstrated that metals associated with PM can exert a proinflammatory effect in the respiratory system and the generation of ROS by transition metals (e.g., Fe, Ni, Cu, Co, and Cr) may play an important role in this effect [29–31]. One study conducted by Becker et al. showed that PM10 stimulated interleukin-8 (IL-8) and ROS production in normal human bronchial epithelial (NHBE) cells and IL-6 production in alveolar macrophages. Further analyses of the principal components indicated that, whereas Fe and Si in the PM correlated with IL-6 release, Cr correlated with IL-8 increase [32]. Soluble metals on inhaled PM have been demonstrated to induce cellular oxidative stress in airway epithelial cells. Using metal chelator and antioxidants, a number of metals have been identified as responsible for the pro-oxidant and proinflammatory effects of PM. High vanadium (V) content in residual oil fly ash (ROFA) has been implicated in the activation of NF-κB and increased production of IL-6 in NHBE cells [33]. PM with high Cu content induced cytokine release and NF-κB activation in a human bronchial epithelial cell line (BEAS-2B) [33]. In animal studies, short-term exposure to CAP aerosol led to a significant increase in thiobarbituric acid-reactive substances (TBARS) and oxidized proteins in rat lung, indicating the presence of oxidative stress. This was accompanied by increased polymorphonuclear cells (PMN) in the bronchoalveolar lavage (BAL) from these animals. A strong association has been identified between increased TBARS and Al, Si, and Fe content of CAP. There was also a correlation between PMN count in BAL and CAP-associated Cr, Zn, and Na [34]. The soluble fraction of ROFA has been shown to be capable of generating metal-dependent hydroxyl radicals in a cell-free system and cause lung inflammation in rats [35]. The pro-oxidative and proinflammatory effects of PM-associated metals have also been demonstrated in human studies. One report showed that instillation of metal-rich ambient $PM_{2.5}$ from a smelter area into the lungs of healthy subjects resulted in airway inflammation characterized by increased ROS and cytokine production (IL-6 and TNF-α), as well as monocyte infiltration. It has been suggested that transition metals may be responsible for these effects [36]. Studies conducted in Utah Valley have provided another piece of evidence demonstrating the role of metals in the biological effects of ambient PM [37]. The closure and opening of a steel mill in the area had significant impact on the PM levels, as well as its composition. When the steel mill was operating, the PM in the area contained significantly larger amounts of metals, including Fe, Cu, Zn, Pb, Ni, and V. Aqueous extracts from the metal-rich PM had stronger ability to generate ROS and to increase IL-8 and IL-6 release by BEAS-2B cells compared to the PM collected when the steel mill was closed. Human exposure to the aqueous PM extracts containing high metal content caused inflammation in the lower respiratory tract as evidenced by significantly increased IL-8 and TNF levels in the BAL fluid. It has been suggested that the inflammatory injury correlated with the metal content and redox potential of the PM [37].

All considered, it could be concluded that redox-active organic chemicals could be major PM toxicants that are responsible for ROS generation and the induction of oxidative stress. Metals may synergize with organic PM components in this process, leading to further escalation of oxidative stress [38].

The impact of particulate pollutants on asthma

Epidemiological evidence has shown a good correlation between increased ambient PM levels and cardiorespiratory morbidity and mortality [22,39]. There is growing recognition that susceptible people could be more prone to these adverse health effects and that the protective effect of Tier 1 of the hierarchical oxidative stress model may be helpful in understanding this susceptibility. This is best explained by studies looking at pro-oxidative and proinflammatory PM effects in the lung.

PM is capable of generating acute airway inflammation that can lead to asthma flares after a sudden surge in ambient $PM_{2.5}$ levels [39–41]. These acute exacerbations are characterized by increased symptom score as well as the requirement for more frequent medication and hospitalization [39,40]. In addition to these acute effects, which are likely caused by an exacerbation of already existing airway inflammation and airway hyperreactivity, there is increasing evidence that particulate pollutants act as an adjuvant for allergic sensitization to common environmental allergens [25,42–46]. This raises the possibility that long-term PM exposures may lead to increased prevalence of asthma and allergic diseases. Although this notion is compatible with the increased prevalence of asthma in polluted urban environments, this topic is still controversial because of the complicated pathogenesis of this disease, including the existence of heterogeneous asthma phenotypes that may be differently affected by environmental stimuli [47].

In addition to the epidemiological evidence, experimental data also suggest an association between particulate pollutants and asthma [1,48]. DEP, one of the major sources of ambient UFP, have been used as a model particulate pollutant to elucidate the mechanisms by which PM may contribute to asthma. This includes some evidence that the generation of oxidative stress by organic DEP chemicals could be responsible for the proinflammatory and adjuvant effects of these particles in the respiratory tract [23,49–51]. Redox-active PM chemicals can exert nonspecific proinflammatory as well as allergic inflammatory effects in the nose depending on whether the challenge is performed in a nonatopic or an atopic individual. Whereas nonspecific inflammation could play a role in acute asthma flares, the adjuvant effects of PM involve the targeting of specific cellular elements in the immune system [1].

In vitro studies have identified macrophages and bronchial epithelial cells as important cellular targets for PM in the lung [50–53]. Exposure of these cells to ambient PM and organic DEP chemicals can induce the generation of ROS and oxidative stress, which can result in increased cytokine and chemokine production [54,55]. Examples include increased TNF-α and IL-6 production in macrophages and IL-8 production in bronchial epithelial cells [54]. Additional evidence supporting the role of oxidative stress in PM-induced airway inflammation comes from animal studies [56–58]. For example, intratracheal instillation of DEP leads to increased PMN infiltration, increased mucus, nitric oxide production, and increased airway hyperreactivity (AHR) in mice, all of which play an important role in the pathogenesis of asthma [59–65]. These effects can be suppressed by pretreating the animals with SOD or with nitric oxide synthase inhibitors [60,63,64]. In addition, thiol antioxidants such as NAC and bucillamine are capable of suppressing the adjuvant effects of aerosolized DEP on ovalbumin (OVA)-induced allergic responses in mice [66]. NAC also abrogated AHR induction by incinerator particles [67]. Using in vivo chemiluminescence imaging, Gurgueira et al. demonstrated that 5-h exposure to CAP aerosol significantly increased ROS production in the lung and heart of Sprague–Dawley rats compared with the animals exposed to filtered air. Increased oxidative stress was accompanied by mild, but significant, damage to both organs [68]. This study has provided the most direct in vivo evidence that PM induce ROS generation and cause oxidative tissue damage. In humans, experimental DEP exposures result in increased CO in exhaled air; CO is the catalytic product of HO-1, which acts as a sensitive marker for PM-induced oxidative stress [69–72].

The adjuvant effects of ambient PM and DEP have been demonstrated in a number of human and animal studies [42–46]. Combined DEP and ragweed nasal challenge significantly enhanced ragweed-specific IgE and IL-4 production in humans [44]. In addition, intranasal instillation of DEP also increased the expression of several CC chemokines, including RANTES, macrophage inflammatory protein-1α (MIP-1α), and macrophage chemoattractant protein-1 (MCP-1), in the human nose [43].

Gilliland et al. reported that individuals with GST M1-null genotype exhibit increased nasal allergic and allergen-specific IgE response to nasal DEP challenge, thereby demonstrating the possible linkage of these responses to an oxidative stress mechanism [73]. This finding suggests that the antioxidant and anti-inflammatory effects of phase 2 enzymes could play an important role in protecting against the proinflammatory and proallergic effects of PM [74–76].

In animal studies, DEP has been shown to enhance OVA-induced eosinophilic airway inflammation, OVA-specific IgG1 and IgE production, goblet cell proliferation, and local expression of several T helper 2 (Th2) cytokines and chemokines [77]. Similar results have also been reported in animals receiving intratracheal instillation of the dust mite allergen Der f, in the presence of DEP [78,79]. Furthermore, when Balb/c mice were exposed to an aerosolized leachate of residual oil fly ash, their offspring demonstrated a significant increase in airway hyperresponsiveness, eosinophilic inflammation, and IgE production in response to sensitization with a suboptimal dose of OVA [80]. Cultured splenocytes from these offspring demonstrated an increased IL-4/IFN-γ ratio, suggesting a skewing toward Th2 immunity [80].

The immunological basis for the adjuvant effects of PM is still improperly understood. Several cell types are involved in allergen sensitization and asthma pathogenesis, including antigen-presenting cells (APC), Th2 lymphocytes, IgE-secreting plasma cells, mast cells, eosinophils, neutrophils, mucus-secreting goblet cells, and smooth muscle and endothelial cells. DEP can directly impact a number of cells that play a role in the afferent or efferent immune response [25,81–87]. Traditional adjuvants exert their effects on the afferent or early phase of the immune response, which implies possible effects on APC [88,89]. Consequently, a lot of attention is currently being directed at the possible contribution of dendritic cells (DC). DC play a crucial role in initiating T cell activation and are the main APC responsible for allergen processing and presentation in asthma. Airway DC continuously sample their environment for antigens and allergens [90–92]. After allergen capture and receipt of a danger signal, DC upregulate CCR7 expression, enter the afferent lymphatic vessels, and carry the allergen to the draining lymph nodes, where it is presented by the major histocompatibility complex in the presence of costimulatory molecules. Allergen-specific T cells are selected for antigen specificity and induced to proliferate. Depending on the cytokine milieu and other variables, DC could initiate a primary Th2 response in regional lymph nodes [90–95]. After immune excitation, memory/effector $CD4^+$ Th2 cells then leave the draining lymph nodes and extravasate at sites of inflammation during the challenge phase. Once in the tissues, Th2 cells interact with IgE-bearing local DC to increase IL-4, IL-5, IL-9, and IL-13 production [90–92,96–102]. These cytokines are important for inducing tissue eosinophilia, airway hyperreactivity, and the production of chemokines that attract further inflammatory cells.

What is the evidence that PM can impact this scenario of events? First, it has been reported that certain PM are capable of skewing the immune response toward Th2 differentiation by interfering with DC function. A recent study demonstrated that diesel-enriched PM could increase antigen uptake by DC while also enhancing the surface expression of costimulatory molecules [103]. In costimulation assays of PM-exposed DC and alloreactive $CD4^+$ T cells, DEP directed a Th2-like pattern of cytokine production (e.g., enhanced IL-13 and IL-18 and suppressed IFN-γ production) [103]. Several studies now seem to indicate that oxidative stress is capable of shifting the immune response from Th1 to Th2 dominance [104]. In this regard, we have demonstrated that organic DEP extracts are capable of inducing oxidative stress effects in myeloid DC that lead to interference in production of IL-12, a key cytokine for T helper 1 immunity [105]. This, in turn, results in decreased IFN-γ production in antigen-specific T cells, which means that the overall decrease in Th1 immunity could promote Th2 skewing of the immune response [106]. Similar effects on IFN-γ production have been demonstrated in intact animals [106]. One possible explanation for the perturbation of DC function by oxidative stress is the activation of the Nrf2-mediated pathway, which exerts negative regulatory effects on the NF-κB signaling [105]. The NF-κB pathway plays an important role in IL-12 production, costimulatory receptor expression, and DC maturation. Thus, one scenario is that a decrease in Th1 immunity may promote the adjuvant effects of DEP.

In addition to adjuvant effects, PM exposure induces acute asthma exacerbations independent of their effect on allergic sensitization [107]. For instance, it is capable of inducing AHR in naïve mice in the absence of allergen [62,108]. It has also been demonstrated that DEP alone can induce increased AHR in asthmatic individuals [109]. Whereas these effects may be related to PM effects on the immune system, the particles and their components may directly contribute to increased AHR during asthma attack [110–112]. One possible mechanism is nitric oxide generation, as evidenced by the ability of nitric oxide synthase inhibitors to interfere with DEP-induced AHR in mice [60]. Shedding of airway epithelial cells is another possibility, based on the ability of DEP to induce acute epithelial damage in vivo and in vitro [51,113–115].

Two recent reviews have summarized the potential mechanisms of PM–lung interaction and particle translocation to other tissues with a focus on the UFP [116,117]. It has been suggested that the unique physical and chemical properties of UFP play important roles in particle deposition in the lung and translocation to the extralung tissues. When inhaled UFP deposit on the epithelial surface of the peripheral lungs, their contact with the surfactant layer and epithelial lining fluid (ELF) leads to their interactions with proteins and other biomolecules in the ELF. The large numbers of UFP, compared with that of micrometer-sized PM, allows them to deposit over a large surface area of alveoli. This may result in a scattered chemoattractant signal that leads to less recognition and phagocytosis of UFP by alveolar macrophages. In addition, PM may form complexes with proteins in the ELF. Whereas proteins on the surface of micrometer-sized PM are immobilized and therefore allow rapid phagocytosis by alveolar macrophages, the extremely small size of UFP may make UFP–protein complexes protein-specific and less accessible to the cells of the defense system, such as macrophages, in the lung

Table 2
Comparison of ambient ultrafine particles (UFP) and nanomaterials

Particle type	Ambient UFP	Nanoparticle
Source	Anthropogenic	Engineered
Size	<100 nm	<100 nm
Uniformity	No	Yes
Organic chemicals	High	Low
Transition metals	High	Varies
Oxidative stress	Yes	Varies
Toxicity	Yes	Varies
Portal of entry	Lung	Lung, skin, blood

epithelium. Modifications of UFP may also allow DC to process these particles, take up antigenic material, and carry it to the immune system, where it elicits an immune response [116,117].

Potential health effects of engineered nanoparticles

In addition to the inadvertent generation of air pollution particles by the burning of fossil-fuel products, the rapid expansion of nanotechnology may lead to adverse health effects. Nanotechnology broadly refers to the manipulation and manufacture of materials and devices in the size range 1–100 nm. These engineered nanomaterials include nanoparticles, nanospheres, nanotubes, and nanofibers. Whereas engineered NP are in the same size range as ambient UFP, they have their own unique physical and chemical characteristics as well as functionality (Table 2). Nanomaterials are used in a wide range of industries, including food, clothing, automobile manufacture, electronics, cosmetics, medicine, and agriculture [3].

Increasing production and usage of nanomaterials in consumer products may lead to human exposures. Among the different exposure routes (e.g., inhalation, skin contact, ingestion, and injection), particle inhalation is an important exposure mechanism. This could happen during manufacturing, shipping, or handling of nanoparticles, especially of those that are produced in bulk or powder form. However, exposure could also happen through the wear and tear of the finished product, e.g., shedding of particles from car tires that include nanomaterials such as carbon nanotubes. Although to date there have been no examples of lung pathology in humans due to the inhalation of engineered nanoparticles, copious

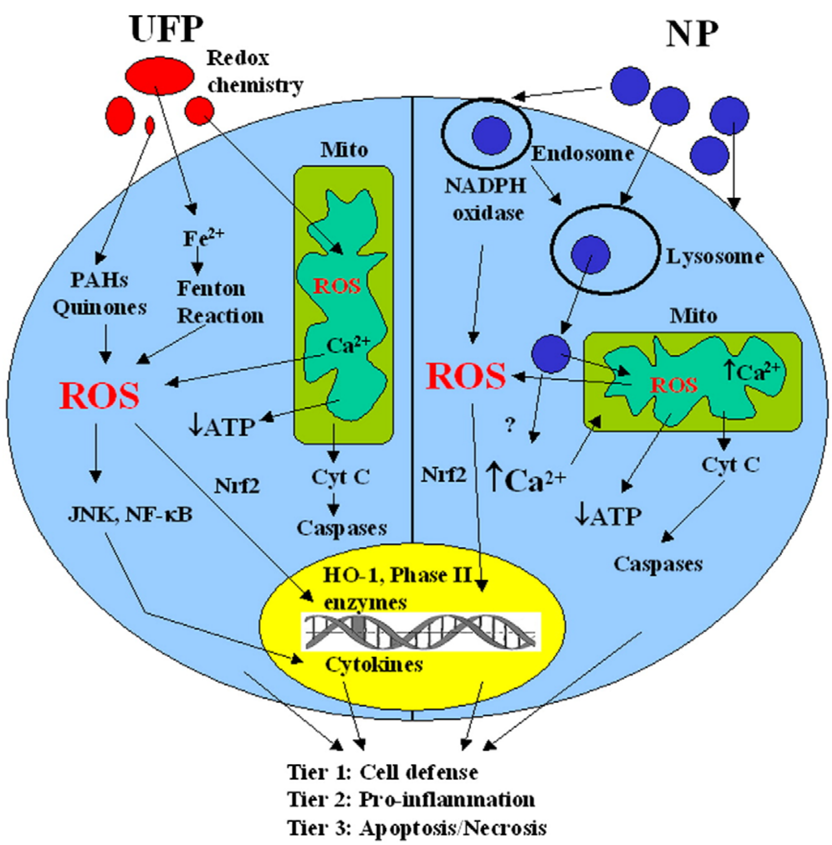

Fig. 2. Comparison of the mechanisms of ROS generation induced by UFP and NM out- or inside of cells. Ambient UFP usually contain large amounts of organic chemicals such as PAH and quinines and transition metals such as Fe and Cu, which can generate ROS through redox chemistry both out- and inside of cells. UFP have also been found to lodge in mitochondria, causing damage to mitochondrial function and structure, which can also produce more ROS. Cells under oxidative stress will have tiered responses including cell defense (Tier 1), proinflammation (Tier 2), and mitochondria-mediated cell death (Tier 3). NM are uniform in size and can also generate ROS via crystal-structural defects or under UV conditions. NM are taken up into cells via endocytosis, which includes phagocytosis, clathrin-dependent endocytosis, caveolae-mediated endocytosis, or macropinocytosis depending on specific cell types. After cells take up NM, endosomes are formed, and ROS can be produced via the formation of NADPH oxidase. After a series of fusion and fission processes, endosomes will fuse with lysosomes. NM can break loose from lysosomes and interact with other organelles such as mitochondria, which can produce more ROS. The cells under oxidative stress will go through tiered oxidative stress responses as described previously.

experimental evidence has been provided for the generation of pulmonary inflammation and interstitial fibrosis by metal oxide nanoparticles and carbon nanotubes, respectively. Thus, the potential exists that nanoparticles could lead to lung disease in humans. Because the understanding of the toxic potential of NP is very limited, nanotoxicology is a new area of science that is looking at the possibility that the novel physicochemical properties of nanomaterials could give rise to hereto-unseen adverse biological outcomes [3,118–120].

Among the possible mechanisms of NP-induced injury, ROS generation remains an important consideration [3,48]. It may be useful therefore to compare engineered to ambient NP in terms of similarities, as well as differences, in the generation of oxidant injury (Table 2, Fig. 2). For example, similar to ambient UFP, some engineered NP are capable of abiotic ROS generation. The proposed NP properties that could lead to this outcome are depicted in Fig. 3. The first is the formation of electron hole pairs in TiO_2 NP by UV activation. The distribution of some of the hole pairs to the particle surface could participate in the electron donor or capture interactions that generate superoxide ($O_2^{\bullet-}$) or hydroxyl radicals ($OH^{\bullet}$), respectively (Fig. 3, upper left quadrant). A second mechanism could be that an excited energy state in a semiconductor NP could lead to an electron jumping from the conduction band to O_2 to generate $O_2^{\bullet-}$ (Fig. 3, upper right quadrant). Examples of such materials include fullerenes and TiO_2. A third mechanism is the dissolution of NP and release of metal ions (e.g., $ZnO \rightarrow Zn^{2+}$) that catalyze ROS generation (Fig. 3, lower left quadrant). Finally, transition metals on the nanomaterial surface (e.g., Fe^{2+} on carbon NT or metal NP) can generate $O_2^{\bullet-}$ via the Fenton reaction (Fig. 3, lower right quadrant). Thus, similar to ambient UFP, ROS generation by nanomaterials could lead to possible adverse biological effects through an oxidant injury mechanism. The magnitude, localization, and site of tissue injury will depend on where the exposure to the nanomaterials takes place. ROS generation by the particle can lead to protein, lipid, and membrane damage [3]. In addition, once the NP are taken up into the cell, their interaction with subcellular organelles and biological systems can lead to further ROS production (Fig. 2) [3,21,121]. One example is disruption of one-electron transfers in the mitochondrial inner membrane. Thus, in addition to the intrinsic properties of the material that could generate ROS, additional nanomaterial properties that are responsible for biological interactions could contribute to further ROS production. It is possible, therefore, that engineered NP devoid of semiconductor properties, UV activation, or transition metals can give rise to ROS generation by perturbing mitochondrial function. For example, cationic polystyrene nanospheres have been shown to induce lysosomal leakage, ROS (H_2O_2 and $O_2^{\bullet-}$) production, and mitochondrial damage, which can eventually lead to apoptosis of murine macrophages [121]. This is an example of an inert material that does not give rise to spontaneous ROS production, yet is capable of inducing ROS production under biological conditions based on the ability of the nanospheres to target mitochondria.

Due to the recent introduction and rapid development of nanotechnology, controlled epidemiological studies on the adverse health effects of NP are basically nonexistent. Animal models for investigating NP toxicity are currently being developed and the number of reports demonstrating the proinflammatory effects of NP in the lung is increasing. For example, intratracheal instillation of a low dose of ultrafine colloidal silica particles (UFCS) elicited moderate to severe pulmonary inflammation and

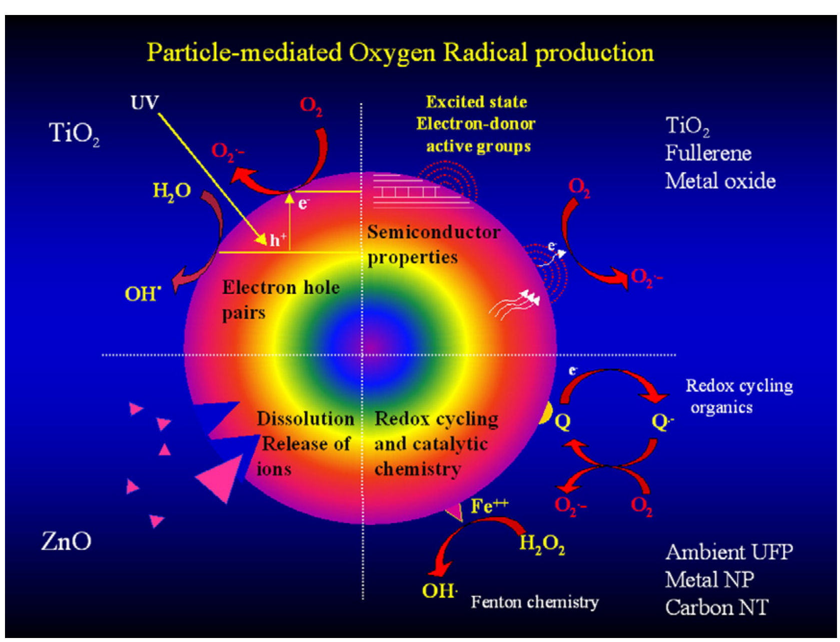

Fig. 3. Nanomaterial (NM) surface properties that are responsible for ROS generation. The valence and conductance bands of semiconductor NM can generate electronic states that lead to the formation of $O_2^{\bullet-}$, which through dismutation or Fenton chemistry is capable of generating additional ROS. Additionally, photoactivation of TiO_2 could generate electron hole pairs that generate $O_2^{\bullet-}$ and $OH^{\bullet}$ radicals. Transition metals and redox-cycling organic chemicals on the particle surface can also participate in ROS generation. Dissolution of the particle surface with the release of metal ions could be particularly relevant to ZnO particle toxicity. These dissolution characteristics could vary with the free surface energy of the particles as well as the pH of the environment or the cell.

tissue injury in ICR mice. Whereas UFCS induced moderate lung inflammation during the acute phase, a significant increase in the apoptotic index could be seen in the lung parenchyma at all times. These lesions correlate with the induction of 8-hydroxyguanosine (8-OHdG) as an oxidative stress marker in lung epithelial cells and activated macrophages [122]. Subacute exposure of C57B1/6 mice to 2- to 5-nm TiO_2 NP in a whole-body exposure chamber caused a moderate but significant inflammatory response in the lung within the first 2 weeks of exposure, beyond which the inflammation resolved without permanent damage [123]. Inoue et al. have demonstrated that intratracheal administration of 14- and 56-nm carbon black NP induced slight lung inflammation and significant pulmonary edema compared with the vehicle [124]. However, when 14-nm carbon black NP were coadministered with bacterial endotoxin, these particles intensively aggravated LPS-induced lung inflammation and pulmonary edema [124]. This pathology was accompanied by increased expression of proinflammatory cytokines such as IL-1β, MIP-1α, MCP-1, MIP-2, and keratinocyte chemoattractant. The level of 8-OHdG in the lung was increased by the nanoparticles independent of the effects of lipopolysaccharide, suggesting that engineered NP could promote the effects of other environmental or inhaled stimuli [124]. The same group also investigated the effects of repeated pulmonary exposure to carbon NP on the expression of a variety of cytokines in the absence or presence of OVA in ICR mice. These studies have also shown that pulmonary exposure to carbon NP induced the expression of thymus and activation-regulated chemokine (TARC), GM-CSF, and MIP-1α in the lung in the absence of OVA. However, in the presence of OVA, the NP considerably enhanced the expression of TARC, GM-CSF, MIP-1α, IL-2, and IL-10. This enhancing effect was inversely related to the particle size [124]. These authors also went on to show that engineered NP may exert adjuvant effects on OVA-related airway inflammation, similar to what we have shown for ambient PM. This adjuvant effect resulted in exaggerated eosinophil, neutrophil, and mononuclear cell infiltration, as well as an increase in OVA-specific IgG and IgE production. The combination of NP with OVA also increased the formation of 8-OHdG and the production of IL-5, IL-6, IL-13, eotaxin, MCP-1, and RANTES in the lung compared with OVA alone [124].

All considered, the available data suggest that engineered NP may contribute to pulmonary morbidity by eliciting proinflammatory effects in the lung and/or by acting as an adjuvant for allergic inflammation. It is possible, therefore, that through the elicitation of an oxidative stress mechanism, engineered NP may contribute to proinflammatory disease processes in the lung. There is no evidence at this stage, however, that engineered NP is contributing to any known human pulmonary disease. Understanding the link between particle-induced oxidative stress and inflammation provides us with a toxicological paradigm on which to base the toxicity screening of engineered NP.

Conclusions

It has been established that there is a close association between exposure to ambient particulate pollutants and increased cardiorespiratory morbidity and mortality. Among the ambient particles, the pulmonary effects of PM_{10} and $PM_{2.5}$ have been more extensively studied than the effects of UFP. However, given the physical and chemical properties of UFP, it is very likely that these particles are more dangerous from the perspective of oxidant injury and inflammation than larger sized particles. Moreover, rapid expansion of the field of nanotechnology has introduced the potential that engineered NP may also become airborne and may contribute to pulmonary disease by novel mechanisms of injury that could include oxidant injury. Thus, the potential exists that these materials could contribute to adverse health effects and we need to take that into consideration when developing methods to screen for NP toxicity. The oxidative stress paradigm currently constitutes one of the best toxicological paradigms on which to base the screening for NP toxicity. However, novel paradigms for injury should also be considered.

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